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## **Influence of knee joint position and sex on vastus medialis regional architecture**

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### **ABSTRACT:**

Ultrasound imaging was used to investigate vastus medialis architecture in 10 males and 10 females at different knee angles. Increase in muscle thickness occurs predominantly when the knee angle is changed from 0° (full extension) and 45° ( $p<0.05$ ); increases in VM pennation angle can be predominantly observed between 45° and 90° ( $p<0.05$ ). Sex differences in the VM architecture can be observed in the distal ( $p<0.01$ ), but not in the proximal region of the muscle ( $p>0.11$ ).

**KEYWORDS:** Ultrasound, Vastus Medialis, Architecture, Sex differences, Anatomy

**ABBREVIATION LIST:** VM: Vastus Medialis; VMD: Vastus Medialis, Distal; VMP: Vastus Medialis, Proximal;

US: Ultrasound; FOA: Fiber Orientation Angle; PA: Pennation Angle; MT: Muscle Thickness.

## INTRODUCTION

Regional variations of muscle thickness, pennation angle, and fiber orientation across the vastus medialis (VM) are well documented in the literature (Blazevich et al. 2006, Smith et al. 2009, O'Brien et al. 2010), but little is known about the influence of the knee joint angle on these indices. Considering that proximal and distal VM regions have different fiber orientation and distal insertions (Smith et al. 2009), any changes in muscle architecture associated with knee joint angle may occur regionally in the VM. In addition, as insertion of the distal VM on the patella is larger in females compared to males (Engelina et al. 2014a), these changes may also be sex-specific. More information on the effect of knee angle on the VM regional architecture could be useful to improve our understanding of the VM muscle function at different knee angles, in males and females.

The purpose of this study was to investigate how changes in knee joint angle influence the architecture of proximal and distal VM. In addition, we tested whether these changes differed between males and females. This was done using ultrasound imaging at rest, which provides reliable (English et al. 2012, Kwah et al. 2013) and valid (Kwah et al. 2013, Engelina et al. 2014b) estimates of muscle architecture. The primary hypothesis was that muscle architecture would change regionally with the knee angle. The secondary hypothesis was that muscle architecture would differ between the sexes.

## MATERIALS AND METHODS

Twenty healthy, recreationally active individuals aged 19-45 years participated in the study (10 males:  $27.5 \pm 2.8$  years old,  $80.0 \pm 9.8$  kg,  $181.5 \pm 4.6$  cm; 10 females:  $26.5 \pm 3.0$  years old,  $56.4 \pm 6.1$  kg,  $163.4 \pm 5.5$  cm). Using a large effect size of 0.4, power 80% and  $\alpha = 0.05$ , ten participants were needed to detect a significant difference in muscle architecture at different knee angles and locations. For this reason, 10 males and 10 females were recruited to address the secondary hypothesis of sex differences. Participants were excluded if they had a history of knee injury or knee surgery, or a recent illness that limited physical activity. Written informed consent was obtained from the participants; all procedures conformed to the standards set by the latest revision of the *Declaration of Helsinki* and were approved by the Clinical Research Ethics Board at the University of British Columbia. Participants were asked to refrain from exercising for 24 hours prior to testing to avoid the possibility of muscle swelling due to delayed onset muscle soreness which could affect the US images.

Physical activity level was measured using the Tegner score (Tegner and Lysholm 1985). A Biodex dynamometer (System 4 Pro; Biodex Medical Systems, Shirley, NY) was used to position the right knee at three angles ( $90^\circ$ ,  $45^\circ$  and  $5^\circ$ ,  $0^\circ$  being full extension) in a randomized order. In each session, one of two investigators

(randomly chosen before starting participant recruitment) obtained the US images. Two recent systematic reviews showed high reliability and validity of ultrasound muscle architecture measurements (English et al. 2012, Kwah et al. 2013). US images were obtained at rest using a B-mode US device (Telemed, Vilnius, Lithuania) with coupling gel (Aquasonic 100, Parker Laboratories INC, NJ, USA) applied on the transducer. Images representative of a proximal region of the VM (VMP) were taken at 30% (based on the midpoint of previous literature (Blazevich et al. 2006, O'Brien et al. 2010)) of the length of a reference line connecting the anterior superior iliac spine to the proximal edge of the patella; the probe was placed just medial to the border between the VM and the rectus femoris, located using US. A second reference line was drawn 25mm medially, parallel to the first one. Images representative of a distal region of the VM (VMD), were taken with the probe positioned medially to this line and aligned to the proximal edge of the patella, palpated at each knee angle (fig.1A). Alignment of the probe to the muscle fiber orientation was obtained by rotating the probe until VM fascicles could be observed running continuously across the screen (Engelina et al. 2014a, 2014b). When this alignment was obtained, an image was saved for offline analysis of pennation angle (VMP and VMD), muscle thickness (VMP only; see below) and fiber orientation angle (FOA). For all images, care was taken to maintain the probe perpendicular to the skin and to apply as little pressure as possible to limit deformation of muscle fascicles. Due to the anatomy of the VMD, muscle thickness cannot be estimated from images obtained with the probe aligned with the fiber orientation (Blazevich et al. 2006). For this reason, the probe was rotated around its longitudinal axis until the superficial and deep VM fasciae were parallel, and a second set of images were collected from VMD. Each measure was taken 3 times, resulting in 9 images (3 for VMP and 6 for VMD) for each knee angle tested.

Ultrasound images were imported, digitized and measured using ImageJ (National Institutes of Health, Bethesda, Maryland, USA). Muscle thickness (MT) was measured at the midpoint of the image, as the distance between the VM superficial and deep aponeurosis (Fig.1B). Pennation angle (PA) was measured as the angle between the most distinct and continuous VM muscle fascicle and the VM deep aponeurosis (Fig.1B and 1C). The fiber orientation angle (FOA) was measured by applying a 20cm linear adhesive foam tape parallel to the ultrasound probe; the angle of intersection between the foam tape and the reference line connecting anterior superior iliac spine and the proximal edge of the patella was measured by goniometer and recorded (Engelina et al. 2014a). Measures of MT, PA and FOA were averaged for the three images taken at each location.

Data were analysed using SPSS v.22.0 (International Business Machines Corp, Armonk, New York). To ensure that physical activity was comparable between males and females, Tegner scores were compared

using the Wilcoxon test. Separate 3-way ANOVAs were used to test the effect of sex, knee angle and site on FOA, MT and PA. Knee angle and site were considered within-subject comparisons. Post-hoc analyses were performed with Bonferroni adjustment for multiple comparisons. All data met the assumptions of normal distribution. To test for any systematic differences between the two investigators taking the US measurements, pilot data were compared between the investigators with paired t-tests. Statistical significance was set at  $p < 0.05$ .

## RESULTS

The physical activity level was not different between males and females ( $p = 0.12$ ; 25<sup>th</sup>-75<sup>th</sup> percentile: 5-6 for both males and females). Pilot US measures were not different between the two investigators ( $p$  values ranged between 0.17 and 0.79).

Fiber orientation angle was not influenced by knee joint angle ( $p = 0.51$ , fig.2A). There was a significant interaction between site and sex on FOA ( $p < 0.001$ ); that is, males had more obliquely-oriented fibers than females in the distal ( $p < 0.001$ , mean difference  $9.7^\circ$ ), but not in the proximal VM ( $p = 0.53$ , mean difference  $0.9^\circ$ ). For both sexes, FOA was larger for the distal than for the proximal VM ( $p < 0.001$ ; females, mean difference:  $24.4^\circ$ ; males, mean difference:  $35.1^\circ$ ).

Pennation angle increased with knee extension (fig.2B). While the 3-way ANOVA identified a significant interaction between knee joint angle and site ( $p = 0.01$ ), post-hoc testing did not identify any site-specific differences. For both locations, PA was significantly smaller at  $90^\circ$  than both  $45^\circ$  ( $p < 0.001$ ) and  $5^\circ$  ( $p < 0.001$ ), and no differences were identified between  $5^\circ$  and  $45^\circ$  (proximal:  $p = 0.06$ ; distal:  $p = 0.14$ ). Irrespective of the knee angle tested, PA was larger in the distal than in the proximal VM ( $p < 0.001$ ). A significant interaction between site and sex was also identified ( $p = 0.01$ ); PA was larger in males than in females in the distal ( $p < 0.001$ ) but not in the proximal VM ( $p = 0.13$ ).

A significant 3-way interaction (fig.2C) was identified ( $p = 0.05$ ) for MT. MT was always larger in the proximal than in the distal VM ( $p < 0.001$ ). MT was larger in males than females in the distal ( $p = 0.01$ ) but not in the proximal VM ( $p = 0.11$ ). In males, MT of the proximal VM was larger at  $5^\circ$  than both  $45^\circ$  ( $p < 0.01$ ) and  $90^\circ$  ( $p < 0.05$ ), whereas no differences were identified between  $45^\circ$  and  $90^\circ$  ( $p = 1.00$ ); in the distal VM, significance was only observed between  $5^\circ$  and  $90^\circ$  ( $p < 0.01$ ). In females, changes in MT with knee angle were observed in the distal ( $5^\circ$  to  $90^\circ$ :  $p < 0.001$ ;  $5^\circ$  to  $45^\circ$ :  $p < 0.01$ ;  $45^\circ$  to  $90^\circ$ :  $p = 0.88$ ) but not in the proximal VM ( $p > 0.93$ ).

## DISCUSSION

At more extended knee positions, larger VM thickness and pennation angle were observed irrespective of the site of detection and sex. A similar trend for a larger increase in PA between a more flexed knee position and mid-range than nearing full knee extension was previously observed in the vastus lateralis (Fig.2, (Fukunaga et al. 1997)). The results of the current study suggest that changes in muscle architecture can be predominantly observed as an increase of pennation angle in the first half of the range; closer to knee extension instead, the main change is the increase in muscle thickness. The finding of increasing pennation angle and muscle thickness with increasing knee extension limits the ability to compare muscle architecture between studies that investigate the VM at different knee angles. Instead, no changes in muscle fiber orientation were observed in this study. The absence of FOA changes with knee joint angle in the distal VM suggests minor changes in the direction of force production of the distal VM at different knee angles. This suggests that the activation of the distal VM would create a medially-directed force vector on the patella at any knee angle, although the resultant movement may be patellar medial translation in full knee extension and patellar rotation at more flexed knee angles (Lin et al. 2004).

The sex differences in VM architecture observed in the distal region of the VM suggests a smaller medially-directed VM force vector in females compared to males. Previous studies reported that fibers in the distal VM were more obliquely-oriented in males than in females, although this difference was small and did not reach statistical significance (Smith et al. 2009, Engelina et al. 2014a). In this study, we report a significant difference of  $9.7^{\circ}$ . A possible reason for these differences is the location of the probe: Engelina et al. (Engelina et al. 2014a) measured the FOA of the VM fibers inserting in the most distal aspect of the patella, whereas in this study the probe placement was aligned to the base of the patella. As variations in fiber orientation can be observed not only between proximal and distal VM, but also within the distal VM itself (Smith et al. 2009, Gallina and Vieira 2015), values from the two studies are likely representative of two different regions within the distal VM. Interestingly, studies on cadavers reported FOA differences between males and females close to  $10^{\circ}$  for the distal vastus lateralis (Becker et al. 2009). For the VM, it is possible that FOA is similar between males and females for the fibers that insert in the most distal location on the patella, but that the change in fiber orientation when more proximal regions of the VM are considered occurs earlier in females than in males; this may also be related to sex-specific differences in the insertion ratio previously described in the literature (Engelina et al. 2014a). Smaller muscle thickness and fiber orientation in the distal, but not in the proximal region, suggests that the medially-directed VM force vector may be less efficient in females than males. This

may be related to sex-specific patellofemoral joint anatomy and biomechanics, and need sex-specific neural control strategies (Tenan et al. 2013).

Consistently with other studies (Engelina et al. 2014a, Khoshkoo et al. 2016), this study measured physical activity using the Tegner score, which does not account for certain types of physical activity such as strength training. As recent research showed that quadriceps strength training may influence VM fiber orientation (Khoshkoo et al. 2016), future research should take in account this factor when investigating sex differences in VM architectural parameters. Similarly, testing size-matched males and females would provide more information on the role of muscle size on the observed sex differences between males and females.

This study confirmed regional differences in vastus medialis architecture, and showed that sex-specific differences in vastus medialis architecture are observed more so in the distal than the proximal VM. In addition, changes in pennation angle and muscle thickness were predominantly observed in the first and second half of the knee joint range, respectively. This information is important to understand the role of regionalization, joint position and sex on muscle mechanics.

#### **CONFLICT OF INTEREST**

The authors report no conflicts of interest associated with this manuscript.

#### **ACKNOWLEDGEMENTS:**

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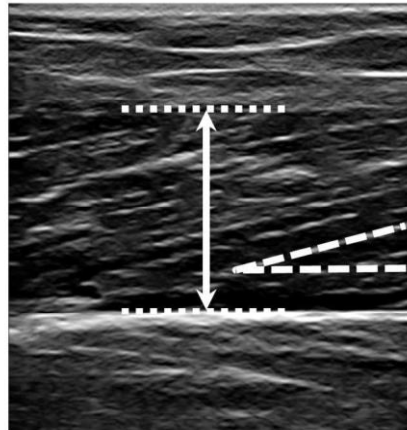
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## FIGURES:

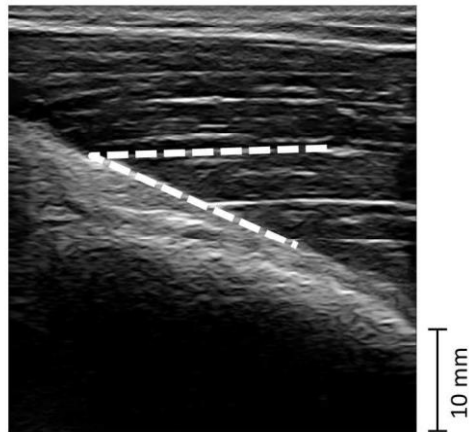
A) Experimental procedure



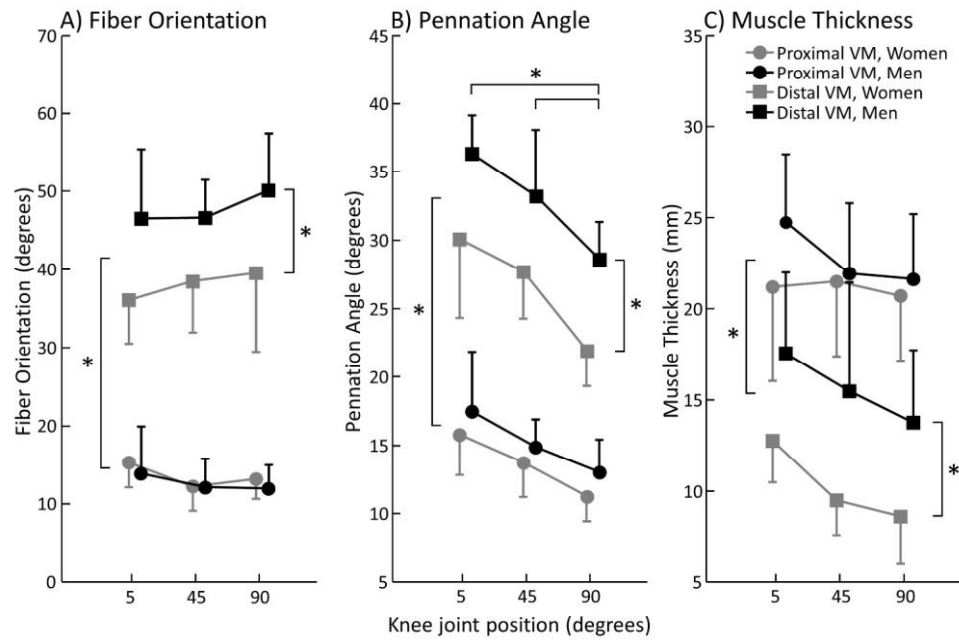
B) Proximal VM



C) Distal VM



**Figure 1.** Experimental protocol (A). Determination of the fiber orientation of the distal VM. The horizontal line identifies the location where the proximal VM was measured (30% of the reference line). Ultrasound scan of proximal (B) and distal (C) VM. Deep and superficial aponeuroses are identified with dotted lines (B). The arrow depicts the measure of muscle thickness. The dashed lines represent measures of pennation angles.



**Figure 2.** Effect of knee joint position, sex and site on VM fiber orientation (A), pennation angle (B) and muscle thickness (C). Mean and SD. Main effects and interactions (location on the left, sex on the right, angle above) are indicated in each plot. Statistical significance is not displayed for angle-specific 3-way interactions for Muscle Thickness. \*  $p < 0.05$